

**THE EFFECT OF ROOT COOLING ON HORMONE  
CONTENT, LEAF CONDUCTANCE AND ROOT HYDRAULIC  
CONDUCTIVITY OF DURUM WHEAT SEEDLINGS  
(*TRITICUM DURUM* L.)**

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**Summary.** The effect of root cooling on hormone content, root hydraulic conductivity and leaf conductance has been investigated in 10-day old seedlings of durum wheat. A decline in root hydraulic conductivity and a drop in relative water content (RWC) was observed. However, RWC of plants with cooled roots increased with time. This recovery of RWC is likely to be due to a decrease in transpiration resulting from stomatal closure. The observed decline in stomatal conductivity cannot be attributed to ABA-induced stomatal closure, since no increase in concentration or delivery of ABA was shown by analysis of xylem sap. A sharp decline in the concentration of cytokinins in shoots was registered shortly after the start of root cooling. The effect is likely to be due to an increase in the rate of cytokinin degradation in the shoot as evidenced by the increase in the activity of cytokinin oxidase. The decrease in shoot cytokinin content might be responsible for inhibition of transpiration, since this hormone is known to maintain stomatal opening, when applied to plants.

**Key words:** cold stress, hormones, *Triticum durum*, water relations

## **Introduction**

Under natural conditions plants experience soil stresses rather frequently. The reactions of plants to soil drought and flooding have been reported repeatedly (Zhang and

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Davies, 1989; Jackson, 1993). Control of leaf growth and transpiration enables survival of plants under conditions of altered water availability. Reaction of leaves to soil stresses implies transfer of signals from roots to shoots. The roles of ABA and ethylene in long distance signaling have been thoroughly studied in case of flooding, drought and soil compacting (Jackson, 2002, Hartung et al. 2002). Much less attention have been paid to local decrease in soil temperature. Plants are frequently subjected to localized root chilling especially early in spring after cold nights when the air temperature increases much quicker than the soil temperature creating a steep temperature gradient between shoots and roots. Such conditions often occur in wheat fields of temperate climates. The responses of stomata to a decrease in air temperature have been studied intensively by Wilkinson et al. (2001). However, the reaction of a plant to a decrease in root zone temperature might be quite different. Accumulation of ABA in shoots of plants was reported shortly after cooling their root to 12°C (Smith, Dale, 1988), which is likely to be important for the control of stomatal conductance. In the field root zone temperatures may be significantly lower. Thus the aim of the present work was to study the effect of root cooling down to 6–7°C on the content of hormones (ABA, IAA and cytokinins) in shoots, roots and root exudate of wheat seedlings in relation to changes in root temperature-dependent water relations. IAA and cytokinins have been analyzed because they are believed to act as antagonists of ABA (Mansfield, McAinsh, 1995). The involvement of cytokinins in reaction to low root zone temperature was reported in earlier publications (Kudoyarova et al, 1998). This contribution describes their significance for the control of water relations under root chilling stress.

## Materials and methods

Spring durum wheat (*Triticum durum* L., cv. Bezenchukskaya 139) plants were grown in water culture. Seeds were germinated in darkness on distilled water supplemented with 50 µM CaCl<sub>2</sub> at a temperature of 24°C for three days. Three-day-old seedlings were transferred to 0.1 strength Hoagland-Arnon nutrient medium and grown under light of 300 mol.m<sup>-2</sup>.sec<sup>-1</sup> and a 14-h photoperiod for 10 days. The day/night air temperature was maintained at the level of 26/20°C, and the temperature of the nutrient medium was 22/18°C. Root zone temperature of 10-d old plants was lowered to about 6°C by adding ice to the nutrient medium. 30 min prior to root cooling, leaves of part of the plants were immersed in 100 µM benzyladenine for 1 min.

Samples for hormone analysis were collected prior to and after the treatment. IAA and ABA were extracted by solvent partitioning. For immunoassays specific rabbit antibodies raised against IAA, ABA and zeatin riboside were used (Veselov et al, 1992; Kudoyarova et al, 1998). Antibodies against cytokinins had high immunoreactivity towards zeatin and its riboside, which were readily separated by thin layer chromato-

graphy prior to immunoassay, and had low cross reactivity to other cytokinins (dihydrozeatin and isopentenyladenine (iPA) derivatives). The immunoassays are described in detail by Kudoyarova et al. (1998).

The rate of transpiration was measured by weighing: 10 seedlings were placed with their roots in a vessel containing 50 ml of nutrient medium, and this vessel was weighed before and after the 15-min-long period of transpiration. The difference reflected the rate of transpiration (evaporation was prevented by covering vessels with a foil). The results of a typical experiment with 5 replicates are presented as means + standard errors.

Relative water content (RWC) was determined by floating leaf pieces on distilled water for 24 h at 22°C in darkness. The turgid weight (TW) was then determined after blotting and the dry weight was determined after the samples have been dried for 24 h at 80°C. Fresh weight (FW), dry weight (DW) and TW were used to determine relative water content

Activity of cytokinin oxidase was determined as described earlier (Vysotskaya et al, 2001).

Stomatal conductivity was measured by means of a porometer (Mk3, Delta-T Devices, UK).

Xylem sap flow of wheat roots was determined by low subatmospheric pressures induced by vacuum and root hydraulic conductivity ( $L_p$  in  $\text{m}\cdot\text{s}^{-1}\cdot\text{Mpa}^{-1}$ ) was estimated as the quotient of water flow at different values of negative pressure (Freudle et al, 1998).

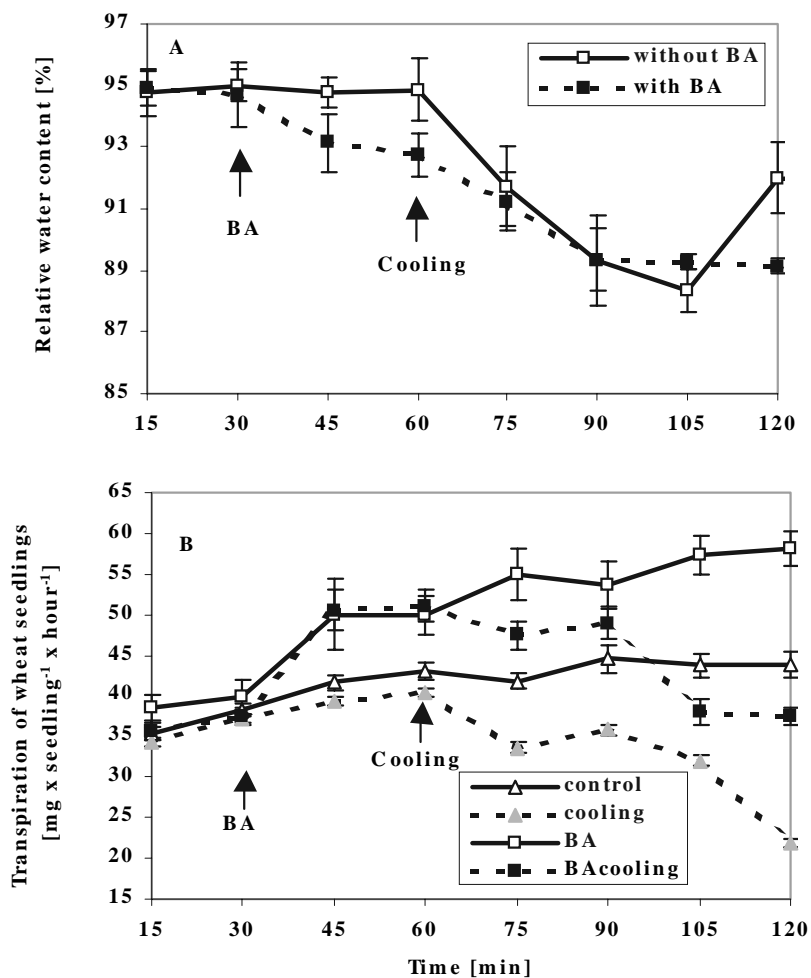
## Results

Measurement of the rate of xylem sap exudation from detached roots at different values of negative pressure showed that root cooling for 30 min caused a decrease in root hydraulic conductivity from 2.4 to  $0.8 \times 10^{-7} \text{m}\cdot\text{s}^{-1}\cdot\text{Mpa}^{-1}$ .

Decrease in root zone temperature initially reduced the relative water content (RWC) of shoots of wheat seedlings (Fig. 1a). However, 105 minutes after the start of the experiment RWC started to recover again. Root cooling reduced transpiration (Fig. 1, b), while pretreatment of leaves with the synthetic cytokinin benzyladenine (BA) prior to root cooling prevented both decline in transpiration and the recovery of RWC (Fig. 1).

Stomatal conductance was 3 mm/s in control, 4.1 mm/s in BA-treated plants, 1.4 and 3.8 mm/s in plants with roots cooled for 30 min (not treated and treated with BA respectively).

The decline in root zone temperature did not influence ABA content in shoots of wheat seedlings significantly, while cytokinin content was two times lower in plants with cooled roots as compared to the control (Table 1). The treatment resulted in



**Fig. 1.** The effect of root cooling to 6–7 °C on RWC (a) and transpiration (b) of 10-d-old wheat seedlings; control (not treated with BA) and treated with BA (30 min prior to root cooling leaves were immersed in 100 μM solution of BA for 1 min).

Table 1. Effect of root cooling for 15 min on hormone content [pmol.g<sup>-1</sup> FW]

	Organ	IAA	ABA	Cytokinins (zeatin+ zeatinriboside)
Control	Shoot	56±3	10.5±0.4	205±22
Control	Root	20±1	5.5±0.9	79±10
root cooling	Shoot	170±9	8.6±0.8	97±8
root cooling	Root	4±0.2	4.3±0.3	83±7

accumulation of IAA in shoots. The effect of root cooling on IAA content in roots was opposite to that observed in shoots: it decreased to an even extent than accumulated in shoots (Table 1). Both ABA and cytokinin content in cooled roots were almost the same as in control. Measurement of ABA concentration in xylem sap did not reveal any significant effect of root cooling (12 and 14 nM in control and treated plants), while calculation of its delivery by multiplying concentration and transpiration rate showed a decline in export of ABA from cooled roots.

The decline in zonal root temperature increased the activity of cytokinin oxidase in shoots (from 410 to 640 pmol iP mg of protein<sup>-1</sup>.h<sup>-1</sup>) 30 min after the start of the treatment by 56%.

## Discussion

Measurement of RWC showed that root cooling increased water deficit in shoots, which is likely to be due to the observed decline in hydraulic conductivity of roots. The latter effect agrees with data of the literature, showing a decline in water uptake at low temperature (Wilkinson et al. 2001). RWC of plants with cooled roots increased with time, which cannot be explained by a recovery of root hydraulic conductivity remaining low when RWC started to increase. Improvement of water relations in treated plants can be better explained by the observed decline in transpiration resulting from stomatal closure, which reduced water losses in accordance with its diminished delivery from cooled roots. Here stomatal closure is not likely to be hydropassive, i.e. caused by an increase in water deficit itself, since results of experiments with plants pretreated with BA give evidence against this assumption. In these plants, root cooling led to an even greater decrease in RWC, while stomatal conductance remained high throughout the experiment. Consequently, stomatal closure in wheat plants with cooled roots is likely to be due to chemical and not hydraulic signals. ABA has been shown in numerous publications to serve as a chemical signal causing stomatal closure in response to root stresses (Jackson, 1993; 2002). However in experiments reported here the concentration of ABA in xylem sap from cooled roots remained the same as in controls, while delivery of the hormone declined. Thus, unlike roots in dry soil, roots cooled to 6–7°C failed to generate an ABA signal. Smith and Dale (1988) reported a rapid increase in ABA content in shoots of plants with roots cooled to 12°C, which was suggested to be due to increased delivery of ABA from roots. However 6–7°C of nutrient medium root zone temperature was likely to be too low in order to enable any increase in the synthetic ability of roots. Thus, ABA cannot be related to decreased stomatal conductance at 6–7°C. These results are in accordance with data in the literature which showed that ABA is not involved in the control of stomatal conductance in plants at low temperature (Wilkinson et al. 2001).

Cytokinins seem to be better candidates for the control of stomatal conductance in wheat plants with cooled roots. According to Tal and Imber (1971) treatment of

plants with exogenous cytokinins kept stomata open. Although attempts to relate changes in the content of endogenous cytokinins to stomatal conductance were not always successful (Incoll, Jewer, 1987), the cytokinin level was likely to be responsible for the control of transpiration when the endogenous ABA was low (Fusseder et al., 1992). Consequently, stomatal closure in plants with cooled roots might be a result of a decrease of cytokinins. This assumption is confirmed by experiments with applied BA, which prevented the reduction in transpiration.

The decline in cytokinin content in the shoots may be a result of a decrease of their export from cooled roots. Delivery of cytokinins from cooled roots was not measured here, since under the conditions of reduced water flow caused by cooling, sampling of xylem sap proved to be extremely difficult. However, some indirect estimates allow the assumption that the observed decline in shoot cytokinins is not solely due to reduced delivery of the hormone from roots. From earlier experiments Kudoyarova et al. (2001) estimated that even after a total stop of root to shoot cytokinin delivery it would take at least 30 minutes to reduce the leaf cytokinin content significantly. In the root cooling experiments of this paper, however, a decrease of cytokinins has been observed 15 minutes after the onset of root cooling. Additional processes must have contributed to the fast drop of shoot cytokinin content. The degradation of cytokinins may be such a mechanism. Indeed root cooling activated cytokinin oxidase. Two factors may modulate the activity of cytokinin oxidase in shoots in response to root cooling. The enzyme activity might be triggered by a root hydraulic signal. There is evidence that changes in cell water potential may influence ABA metabolism (Jackson, 2002) and this might be true for cytokinins as well. On the other hand IAA is known to increase the activity of cytokinin oxidase (Hare, Van Staden, 1994). The accumulation of IAA observed in shoots of wheat seedlings might be responsible for the increase in activity of cytokinins oxidase. Auxin may accumulate in shoots when the export from shoots to cooled roots is reduced. Indeed a sharp drop of IAA in cooled roots could be observed.

Thus, our data suggest that changes in shoot hormones might be responsible for stomatal closure, which balances reduced water delivery from cooled roots.

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